S-11-A-2 Receptor for Granulocyte Colony-Stimulating Factor

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I. INTRODUCTION

Neutrophilic granulocytes have a life-span of only two days in the blood stream, and must be constantly replenished. Neutrophilic granulocytes are produced in bone marrow from the haemopoietic stem cells through several intermediate progenitor cells. Granulocyte colony-stimulating factor (G-CSF) is a member of colony-stimulating factors (CSFs) [1], and specifically regulates proliferation and differentiation of the precursor cells of neutrophilic granulocytes, and can activate mature neutrophils [2,3]. In addition to these physiological activities, G-CSF can induce differentiation of some myelomonocytic leukemia cells to differentiate into granulocytes or monocytes, whereas other myeloid leukemia cells can be stimulated to proliferate [2,3].

In order to understand the molecular mechanism of the G-CSF-triggered proliferation and differentiation of neutrophils, it is important to elucidate the structure and function of the G-CSF receptor. Previously, we have purified murine G-CSF receptor [4], and isolated cDNAs for human and mouse G-CSF receptor [5,6]. Furthermore, mutational analyses of the G-CSF receptor have revealed functional domains of the receptors [7]. In this Symposium, I will summarize current information on the G-CSF receptor.

II. BIOCHEMICAL PROPERTIES OF THE G-CSF RECEPTOR

The G-CSF receptor is present in precursors and mature neutrophilic granulocytes as well as in myeloid leukemia cells which respond to G-CSF [4]. In bone marrow or spleen cells, the receptors are essentially restricted to cells of neutrophilic granulocyte lineage, which agrees with the fact that G-CSF is a specific growth and differentiation factor for neutrophilic granulocytes. The number of receptors is around 300~1,000 per cell, and G-CSF binds to its receptor with a dissociation constant of 100~500 pM [4]. To characterize the biochemical properties of the G-CSF receptor, the G-CSF receptors were purified from NFS-60 cells about 100,000-fold by column chromatography on a G-CSF-affinity gel, and gel filtration. The purified G-CSF receptor had a Mr. of 100,000 to 130,000, and stoichiometrically bound G-CSF. A Scatchard analysis of the binding data indicated that there are two forms of the receptor, one with a high affinity ($K_d=120\sim360$ pM) and the other with a low affinity to G-CSF ($K_d=2.6\sim4.2$ nM). Analysis of the purified receptor by sucrose density gradient centrifugation indicated that the dimer of the receptor has a high affinity for G-CSF

while its monomer binds G-CSF with a low affinity. Since the dissociation constant observed with the receptor dimer is almost identical to that obtained with the cell surface receptor, it was suggested that the G-CSF receptor in the cell surface exists as a homodimer to constitute a high affinity binding site for G-CSF.

III. MOLECULAR AND GENETIC PROPERTIES OF THE G-CSF RECEPTOR

The cDNA for murine G-CSF receptor was isolated from a cDNA library of murine NFS-60 cells by expression cloning [5]. When the cDNA was expressed in monkey COS cells, the cells bound G-CSF with the high affinity observed with the native receptor in NFS-60. These results confirmed that the single polypeptide, as a homodimer, is sufficient to form a specific high affinity binding site for G-CSF.

The murine G-CSF receptor consists of 837 amino acids of which the first 25 amino acids at the N-terminus seem to be a signal sequence. It contains a single transmembrane domain which divides the molecule into two regions, the extracellular and the cytoplasmic. The Mr. calculated from the amino acid composition of the receptor is 90,814, and the extracellular region of the G-CSF receptor comprised of a composite structure. As summarized in Fig. 1, the sequence of about 100 amino acids at the N-terminus, has an immunoglobulin-like sequence (Ig-like). The sequence of the following 200 amino acids is related to that of the extracellular region of various cytokine receptors (cytokine receptor homologous domain or CRH domain). Between the CRH domain and the transmembrane region, the G-CSF receptor contains three fibronectin type III domains (300 amino acids). Unlike the receptors for M-CSF, PDGF and EGF, the G-CSF receptor does not contain a domain for tyrosine kinase.

Figure 1. The structure of murine G-CSF receptor. The structure of murine G-CSF receptor is schematically shown. "Ig-like" indicates an immunoglobulin-like structure. In the cytokine receptor homology domain (CRH domain), the four conserved cysteine residues and the Trp-Ser-X-Trp-Ser ("WSXWS") motif are indicated by bars.

G-CSF and IL-6 have a significant similarity at the amino acid sequence level [2]. The IL-6 receptor consists of two polypeptides. The one (α -chain of the IL-6 receptor) having Mr. of 80,000 binds IL-6, but cannot transduce IL-6-triggered signals into cells. To transduce the signal, the α -chain has to associate with the 130 K protein (gp130, β -chain of the IL-6 receptor) [8]. The primary structure of the G-CSF receptor has no significant similarity to the α -chain of the IL-6 receptor except limited homology in the CRH domain. On the other hand, recent cloning and structural analysis of the cDNA for the gp130 molecule has revealed a remarkable similarity between this molecule and the G-CSF receptor [9]. The overall similarity of the gp130 to the G-CSF receptor at the amino acid sequence level is 46.3% including the conservative substitutions of amino acids. Despite this high similarity to the G-CSF receptor, the gp130 of the IL-6 receptor system neither binds IL-6 nor G-CSF.

IV. THE CYTOKINE RECEPTOR FAMILY AND SIGNAL TRANSDUCTION

In the last few years, the cDNAs for various cytokine receptors such as IL-2~7, G-CSF, GM-CSF and erythropoietin have been isolated. All these receptors contain an approximate 200 amino acid domain (CRH) in the extracellular region, which is related to that of growth hormone and prolactin receptors, suggesting that these cytokine receptors constitute a novel family of receptors [10]. In the CRH domain, the four cysteine residues in the N-terminal half, and the Trp-Ser-X-Trp-Ser motif (WSXWS motif) at the C-terminus are rather strictly conserved among members of this receptor family. The cytoplasmic regions of members of this receptor family are not related to each other, and do not contain the domain for kinase activity.

To examine the signal transduction mechanism of the cytokine receptor, we expressed G-CSF receptor cDNA in mouse IL-3-dependent myeloid leukemia cells which do not have the G-CSF receptor [7]. The transformant clones expressing the G-CSF receptor bound G-CSF with a high affinity, and the G-CSF supported cell proliferation. This indicates that the G-CSF receptor encoded by the cloned cDNA is sufficient to transduce the G-CSF-triggered growth signal into cells, and that the G-CSF receptor uses the signal transducing pathway common to that of the IL-3 system.

What kinds of signals are transduced by the cytokine receptor family? A set of proteins was phosphorylated at the tyrosine residue shortly after the stimulation of cells with G-CSF, IL-2 and IL-3 [11]. Introduction of protein kinases such as v-src, v-abl, v-trk and v-fms into IL-3-dependent cells abrogated the dependence of cells on IL-3 [12]. Furthermore, IL-2 specifically stimulates phosphorylation of lck, a src-family tyrosine kinase, suggesting a close association of lck with the IL-2 receptor [13]. Since some members of the src family such as hck or fgr are expressed exclusively in neutrophilic granulocytes and macrophages [14], it is possible that these protein kinases are involved in the signal transducing pathway of the G-CSF/G-CSF receptor system.

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